Supplemental Information

Figure S1. MTT Assays in COS-7 and Neuroblastoma SH-SY5Y Cells Transduced with AAV Virus. **(A)** Overexposure of the Western blot in HEK293T cells. **(B)** Western blot analysis revealed abundant expression of full-length DISC1 (FL), truncated DISC1 (Trunc), and BD13 in COS-7 cells. The expression of DB7 (DISC1-Boymaw) was very low, and restricted to the pellet fraction. Equal amounts of proteins were loaded in Western blot (see β-actin control bands). **(C)** Subcellular localization of the full-length DISC1, truncated DISC1, DB7, and BD13 proteins demonstrated by immunocytochemical staining with anti-HA antibody. Scale bar: 25 µm. Consistent with previous report (89), full-length DISC1 proteins displayed both diffusive and punctate staining patterns. Both truncated DISC1 and Boymaw-DISC1 proteins were predominantly diffusive. The expression of the DISC1-Boymaw proteins was in general very low. In a few cells displaying strong staining, the DISC1-Boymaw proteins appeared in aggregates. **(D)** MTT reduction assays at different time points. There were 4 replica wells per construct per time point. The control pAAV-GFP was used as the reference for the other four gene constructs. ANOVA analysis revealed a significant gene effect (F(4,90)=18.66, $p < 0.0001$). *Post hoc* analysis revealed that cells transfected with the DISC1-Boymaw (DB7) construct displayed a significant decrease in MTT reduction at all time points except 45 hr post-transfection. Error bar: SEM. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). **(E)** Recombinant AAV1/8 virus was generated to express full-length DISC1 (AAV-FL), truncated DISC1, DISC1-Boymaw (DB7), and Boymaw-DISC1 (BD13) proteins respectively. The titers of the virus were around $2x10^{12}$. MTT reduction was measured two days after transduction of neuroblastoma SH-SY5Y cells in 24 well plates. Each genotype contained 10 to 12 replica for statistical analysis. Error bar: SEM. *** $p < 0.001$. **(F)** Western blot analysis of DISC1-FL, truncated DISC1, BD13, and DB7 proteins in the absence or presence of proteasome inhibitor N-acetyl-L-leucyl-L-leucyl-L-norleucinal (LLnL). The final concentration of LLnL was 40 uM in culture medium. Addition of proteasome inhibitor LLnL remarkably increases the expression level of DB7 protein and the other three proteins in both supernatant and pellet fractions.

Figure S2. iTRAQ Analysis of the Expression of the DISC1-Boymaw Gene in HEK293T Cells. A 4-plex iTRAQ was conducted on the control and test pools. The control pool consists of 9 independent samples transfected with the full-length DISC1 construct, and the test pool consists of 9 independent samples transfected with the DB7 construct. Each pool was labeled with two different mas tags **(A)**. Theoretically, we should expect that the ratio between the two quantifications of the same control pool (115:114) is very close to 1. In fact, there are numerous proteins whose ratios display substantial variation (>1.2, or <0.8) **(B)**. For some reasons, these proteins were not quantified consistently using iTRAQ. Most of these proteins are statistically significant in both the control/control and test/control ratios. Because of the uncertainty of their quantification, these proteins were excluded in the short list **(C)**. Top 18 differentially expressed proteins were listed according to their p values. Proteins highlighted with blue are directly involved in the regulation of protein translation. Our iTRAQ analysis identified 4300 individual proteins (see the full list).

Figure S3. NADH Concentrations. (A) There was no difference in cell proliferation between cells expressing full-length DISC1 (n=10) and DB7 (n=10) genes. **(B)** There was no difference in the concentration of total NADH between cells expressing full-length DISC1 (n=10) and DB7 (n=10) genes. **(C)** pAAV-DISC1-FL and pAAV-DB7 expression constructs were co-transfected with Peredox plasmids (Addgene, Plasmid 32380: GW1-Peredox-mCherry) into HEK293T cells. Green and red fluorescence as well as their ratio were examined two days after transfection. No difference was observed in the green/red ratio between cells transfected with either DISC1-FL/Peredox or DB7/Peredox. Scale bar: 15 µm. **(D)** There was no difference in cell proliferation between cells transfected with DISC1-FL (n=12) and the DB7 (n=12). Reduction of rRNA expression in cells expressing the DB7 was not caused by cell numbers.

Figure S4. Antibody Evaluation and Expression of the Boymaw –DISC1 proteins. (A) 8 different anti-human DISC1 antibodies were initially evaluated with Western blot using HEK293 cell lysate (over-expressing full-length human DISC1) to identify the best antibodies recognizing human DISC1 proteins. Seven antibodies reacted with full-length human DISC1 proteins. ab55808 (abcam), ab55806 (abcam), ab55810 (abcam), ab62069 (abcam), ab59017 (abcam), sc47988 (Santa Cruz Biotechnology), 3D4 and 14F2 (gift from Dr. Carsten Korth). **(B)** Two antibodies (ab55806 and ab59017) recognizing the C-terminal residues of human DISC1 proteins react with HEK293 cell lysate overexpressing the BD13 proteins (containing the C-terminal of human DISC1 proteins) in Western blot. However, antibody against HA epitope generated much stronger signals than the two DISC1 antibodies. **(C)** Antibody ab55806 generated some background in Western blot analysis of BD13 proteins in primary neurons from wildtype and the DISC1-Boymaw homozygous mice. No detectable BD13 was found in the homozygous mice. **(D)** Antibody ab59017 failed to detect BD13 proteins in the homozygous mice.

Figure S5. Nissl Staining of the Heterozygous DISC1-Boymaw Mouse Brain. No gross anatomical abnormalities were found in brain (**A**, **B**, scale bar: 1000 µm), hippocampus (**C**, **D**, scale bar: 500 µm), and cortex (**E**, **F**, scale bar: 130 µm) between adult wildtype (**A, C, E**) and heterozygous DISC1-Boymaw mice (**B, D, F**). Organization and density of hippocampal neurons and cortical layers (1 to 6) are comparable between DISC1-Boymaw heterozygous and wildtype mice.

Figure S6. rRNA Expression and Mouse Endogenous *disc1* Gene Expression. (A) Significant reduction of total RNA was also observed in the cortex of the heterozygous DISC1-Boymaw mice (n=9) in comparison with wildtype mice (n=12) after normalization with DNA (* $p < 0.05$, unpaired, two-tailed student's t-test). No difference was observed in DNA after normalization with the weight of cortex between the two genotypes. **(B)** RNA *in situ* hybridization with 18S rRNA antisense and sense oligonucleotides. Strong signals were detected with the antisense probes; no signal was detected using the sense probes (Scale bar: 1000 µm). **(C)** In 129S mice, endogenous *disc1* gene carries a frame-shift deletion in exon 6. To examine whether there is any alternative splicing to skip exon 6 to generate different DISC1 protein isoforms, we conducted a series of RT-PCR to examine disc1 mRNA alternative splicing: e2f-e10r (exon 2 to 10): 1003 bp; e3f-e10r (exon 3 to 10): 923 bp; e4f-e10r (exon 4 to 10): 802 bp; e5f-e10r (exon 5 to 10): 652 bp; e6f-e10r (exon 6 to10): 517 bp. No alternative splicing was observed. All amplified cDNA fragments were sequenced to confirm the presence of 25 bp deletion in exon 6. **(D)** Western blot analysis of mouse endogenous Disc1 proteins in C57 and 129S wildtype mice. Mouse monoclonal anti-mouse DISC1 2B3 and rabbit anti-mouse DISC1 440/2 antibodies were kindly provided by Dr. Nick Brandon (Pfizer)(8). Proteins were extracted from cultured primary neurons isolated from P1 postnatal mice. A DISC1 protein band about 100 kD was observed in C57 mice. Neither full length nor truncated DISC1 proteins were detected in 129S mice. These data were consistent with previously published studies (6, 35).

Figure S7. Ketamine Responses of the Heterozygous DISC1-Boymaw Mice on F1 129S/C57 Background. (A) In the F1 generation of male mice, there is a trend of gene X ketamine interaction in counts (F(1,27)=3.30, p=0.08) in the BPM. **(B)** A significant gene X ketamine interaction was observed in transitions (F(1,27)=4.00, p=0.05).

Figure S8. Behavioral Characterization of the Heterozygous DISC1-Boymaw Mice on 129S Background. (A) In comparison with the F1 mice on 129S/C57 background, mice on 129S genetic background have higher and more homogeneous startles between individuals. Black dots are females, and red dots are males. After ketamine administration, the heterozygous male mice displayed prolonged responses to ketamine in counts **(B)**(F(1,26)=8.19, $p < 0.01$), transition **(C)**(F(1,26)=7.27, $p < 0.05$), and corner cross **(D)**(F(1,26)=5.58, $p < 0.05$). **(E)** Injection of ketamine increased locomotion in both genotypes of female mice. No gene X ketamine interaction was found. **(F)** The wildtype male mice displayed no significant preference for saccharin. No significant difference in saccharin preference was detected between the heterozygous DISC1-Boymaw and wildtype male mice.

Figure S9. Co-Localization between the DB7 Fusion Protein and Cytochrome C. COS7 cells were transfected with DISC1-FL, truncated DISC1, BD13, and DB7 expression constructs. 40 hr later, the cells were fixed for immunocytochemical analysis with the following primary antibodies: mouse monoclonal anti-Cytochrome C (1:250), ab110325 (Abcam, MA); rabbit polyclonal anti-HA (1:400), H-6908 (Sigma-Aldrich, MO). The secondary antibodies are: Alexa Fluor® 568 goat anti-mouse IgG (1:500), (Invitrogen, CA) and Alexa Fluor® 488 goat anti-rabbit IgG (1:500), (Invitrogen, CA). The coverslips were mounted with VECTASHIELDR Hard Set™ Mounting Medium with DAPI (Vector, CA). Images were acquired with Olympus FluoView™ FV1000 confocal microscope. Scale bar: 20 µm.

Table S1: Quantification of Differential Protein Expression by iTRAQ Analysis. The full list of proteins and their abundance between HEK293T cells expressing either DISC1-FL or DB7.

Table S2. Complete Blood Cell Analysis of Individual Adult Male Mice . Hematological analysis was conducted using the Hemavet 950 FS at the UC San Diego Hematology Core Laboratory. The analysis was duplicated for each blood sample, and the mean values after duplication were used for further analysis. There was a significant increase in RDW of the heterozygous DISC1-Boymaw mice in the analysis of the first cohort of male mice (N=6 for each genotype). To avoid type I error by multiple comparisons, we replicated the study using another group of sibling male mice (N=4 per genotype). Increased RDW in the heterozygous DISC1-Boymaw mice was confirmed. Both studies are combined in this table.

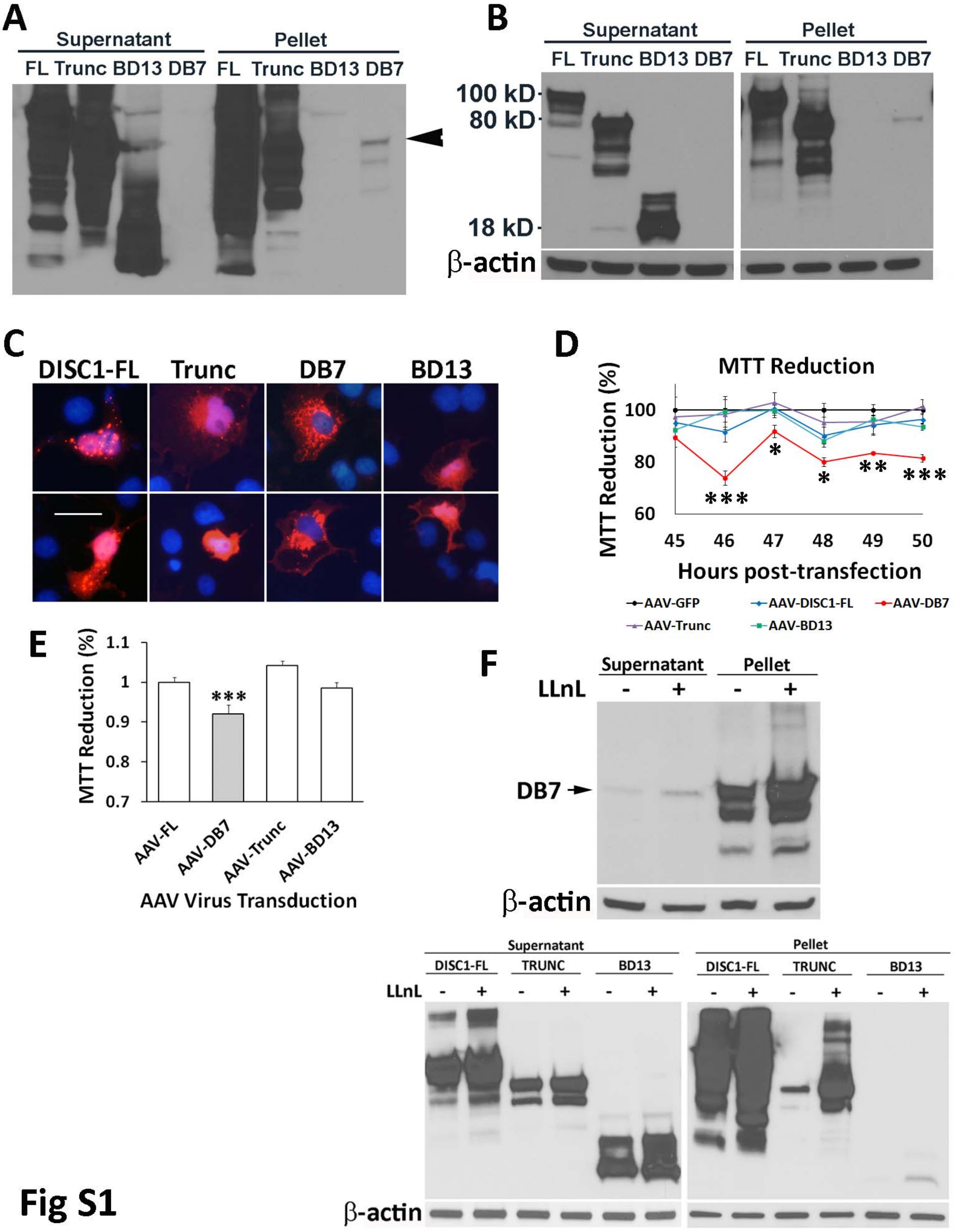
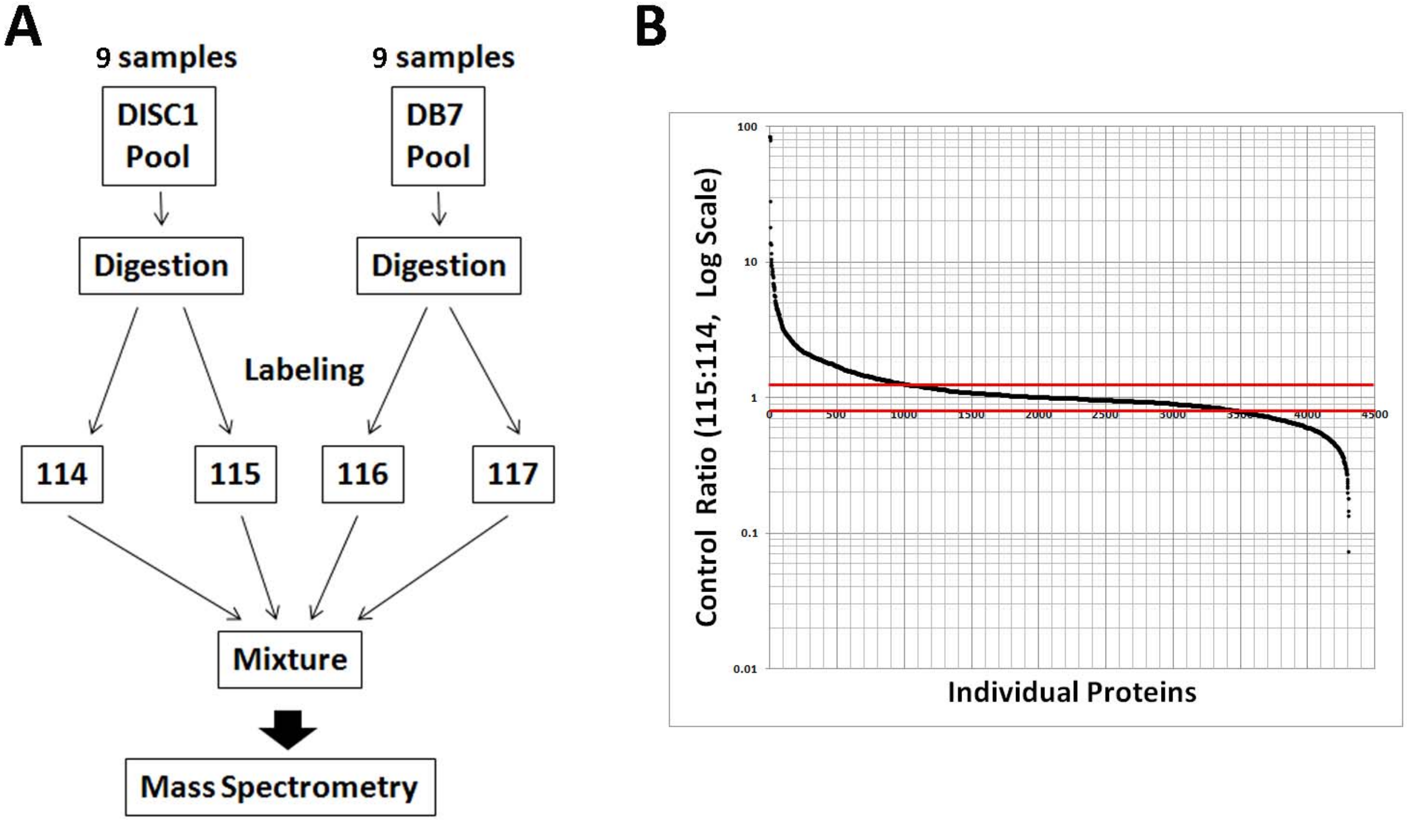
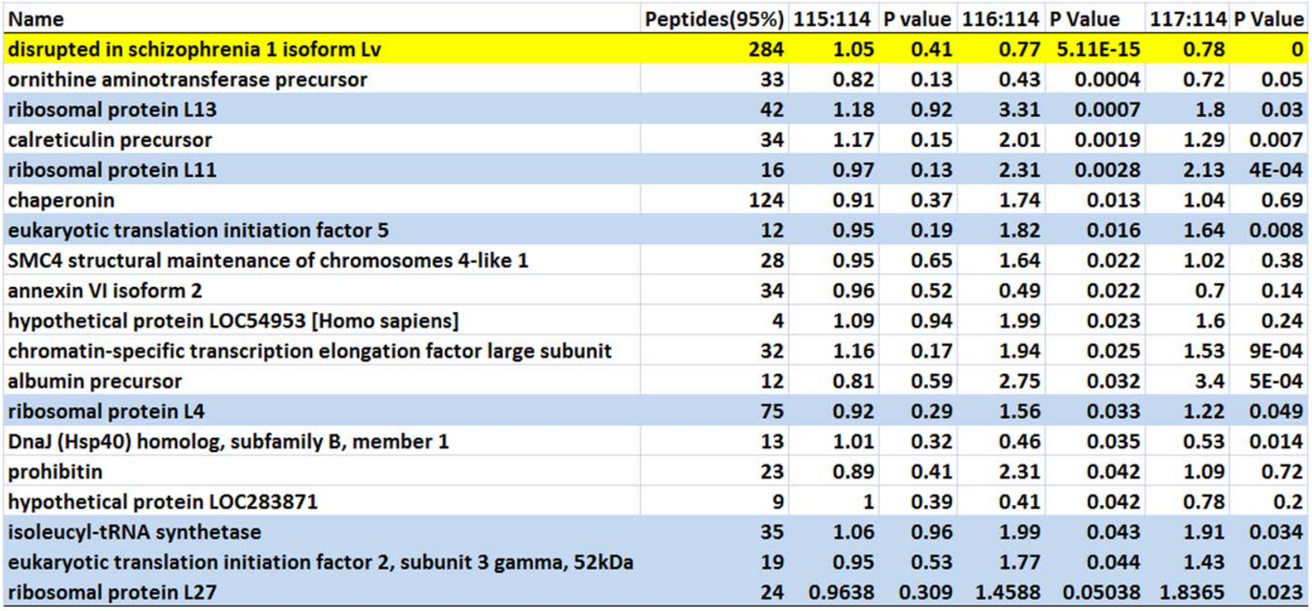

A
Supernatant
Pellet
FL Trunc BD13 DB7
FL Trunc BD13 DB7
B
Supernatant
Pellet
FL Trunc BD13 DB7
FL Trunc BD13 DB7
100 kD
80 kD
18 kD
β-actin
C
DISC1-FL
Trunc
DB7
BD13
D
MTT Reduction
MTT Reduction (%)
100
80
60
45
46
47
48
49
50
Hours post-transfection
***
*
*
**
***
AAV-GFP
AAV-DISC1-FL
AAV-DB7
AAV-Trunc
AAV-BD13
E
MTT Reduction (%)
1.1
1
0.9
0.8
0.7
***
AAV-FL
AAV-DB7
AAV-Trunc
AAV-BD13
AAV Virus Transduction
F
Supernatant
Pellet
LLnL
- + - +
DB7
β-actin
Supernatant
Pellet
DISC1-FL
TRUNC
BD13
DISC1-FL
TRUNC
BD13
LLnL
- + - + - + - + - + - +
β-actin

Fig S1



C



Name	Peptides(95%)	115:114	P value	116:114	P Value	117:114	P Value
disrupted in schizophrenia 1 isoform Lv	284	1.05	0.41	0.77	5.11E-15	0.78	0
ornithine aminotransferase precursor	33	0.82	0.13	0.43	0.0004	0.72	0.05
ribosomal protein L13	42	1.18	0.92	3.31	0.0007	1.8	0.03
calreticulin precursor	34	1.17	0.15	2.01	0.0019	1.29	0.007
ribosomal protein L11	16	0.97	0.13	2.31	0.0028	2.13	4E-04
chaperonin	124	0.91	0.37	1.74	0.013	1.04	0.69
eukaryotic translation initiation factor 5	12	0.95	0.19	1.82	0.016	1.64	0.008
SMC4 structural maintenance of chromosomes 4-like 1	28	0.95	0.65	1.64	0.022	1.02	0.38
annexin VI isoform 2	34	0.96	0.52	0.49	0.022	0.7	0.14
hypothetical protein LOC54953 [Homo sapiens]	4	1.09	0.94	1.99	0.023	1.6	0.24
chromatin-specific transcription elongation factor large subunit	32	1.16	0.17	1.94	0.025	1.53	9E-04
albumin precursor	12	0.81	0.59	2.75	0.032	3.4	5E-04
ribosomal protein L4	75	0.92	0.29	1.56	0.033	1.22	0.049
DnaJ (Hsp40) homolog, subfamily B, member 1	13	1.01	0.32	0.46	0.035	0.53	0.014
prohibitin	23	0.89	0.41	2.31	0.042	1.09	0.72
hypothetical protein LOC283871	9	1	0.39	0.41	0.042	0.78	0.2
isoleucyl-tRNA synthetase	35	1.06	0.96	1.99	0.043	1.91	0.034
eukaryotic translation initiation factor 2, subunit 3 gamma, 52kDa	19	0.95	0.53	1.77	0.044	1.43	0.021
ribosomal protein L27	24	0.9638	0.309	1.4588	0.05038	1.8365	0.023

Fig S2

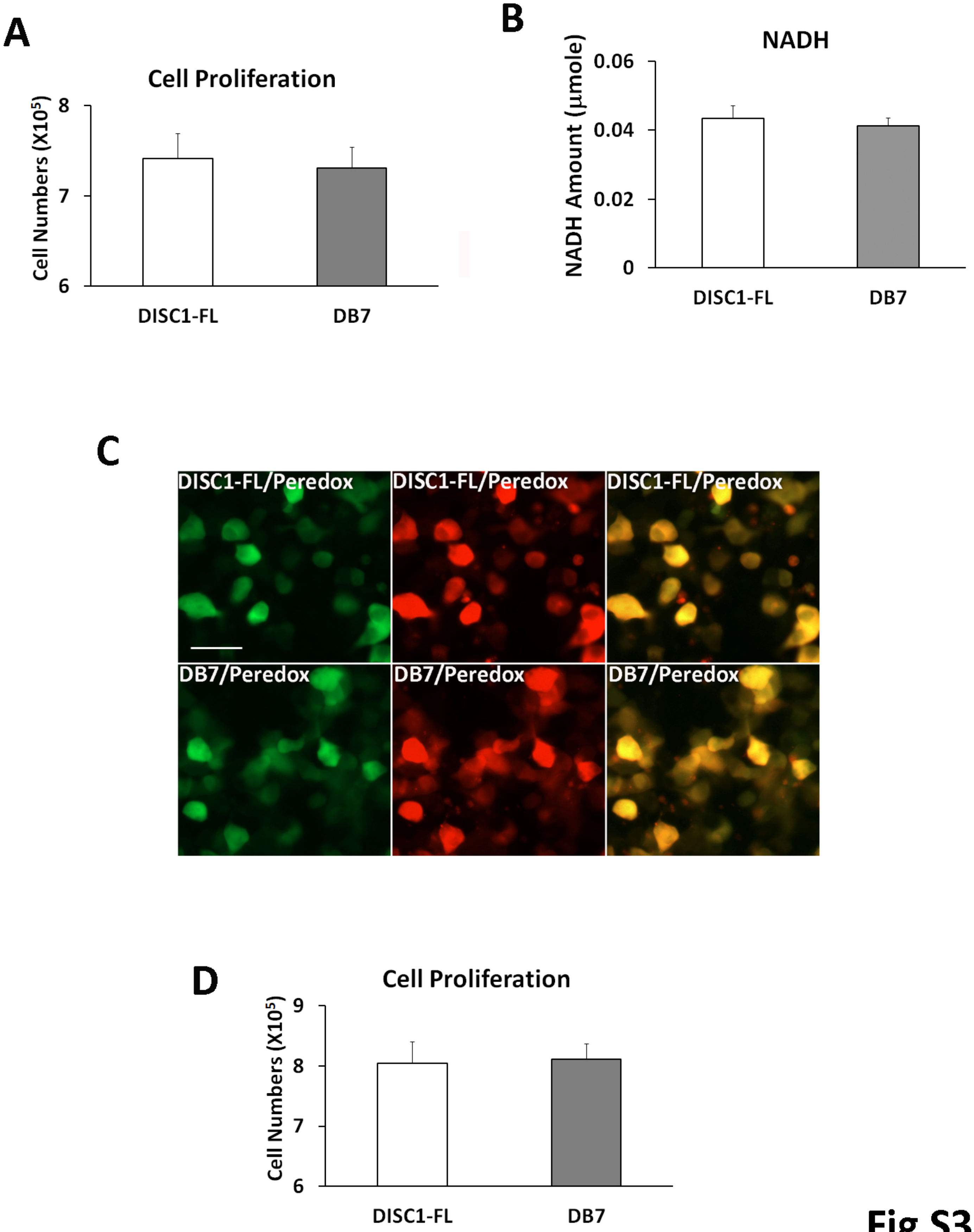

A
Cell Proliferation
Cell Numbers (X10^5)
8
7
6
DISC1-FL
DB7
B
NADH
NADH Amount (µmole)
0.06
0.04
0.02
0
DISC1-FL
DB7
C
DISC1-FL/Peredox
DISC1-FL/Peredox
DISC1-FL/Peredox
DB7/Peredox
DB7/Peredox
DB7/Peredox
D
Cell Proliferation
Cell Numbers (X10^5)
9
8
7
6
DISC1-FL
DB7

Fig S3

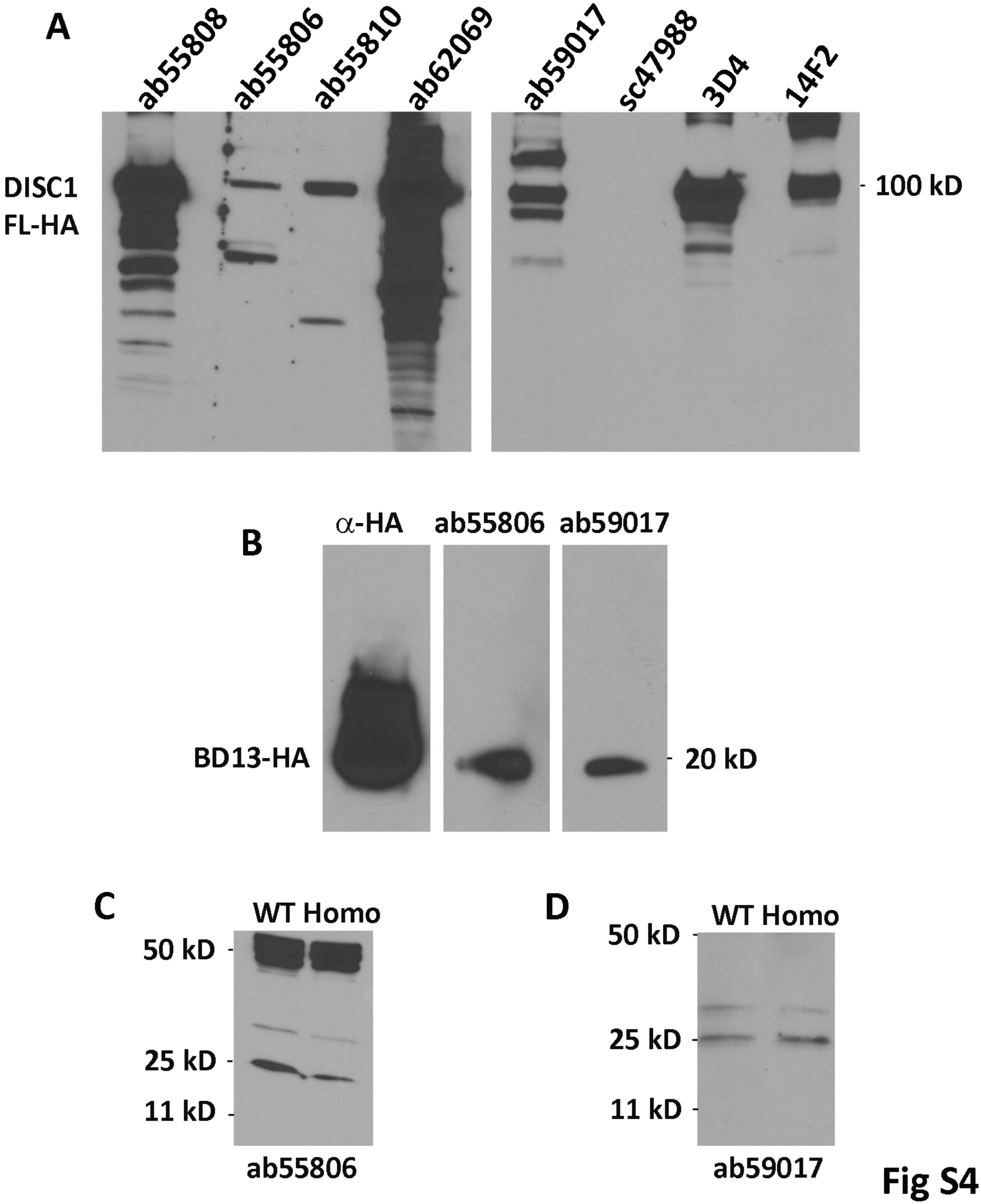

A
ab55808
ab55806
ab55810
ab62069
ab59017
sc47988
3D4
14F2
DISC1
FL-HA
100 kD
B
α-HA
ab55806
ab59017
BD13-HA
20 kD
C
WT Homo
50 kD
25 kD
11 kD
ab55806
D
WT Homo
50 kD
25 kD
11 kD
ab59017

Fig S4

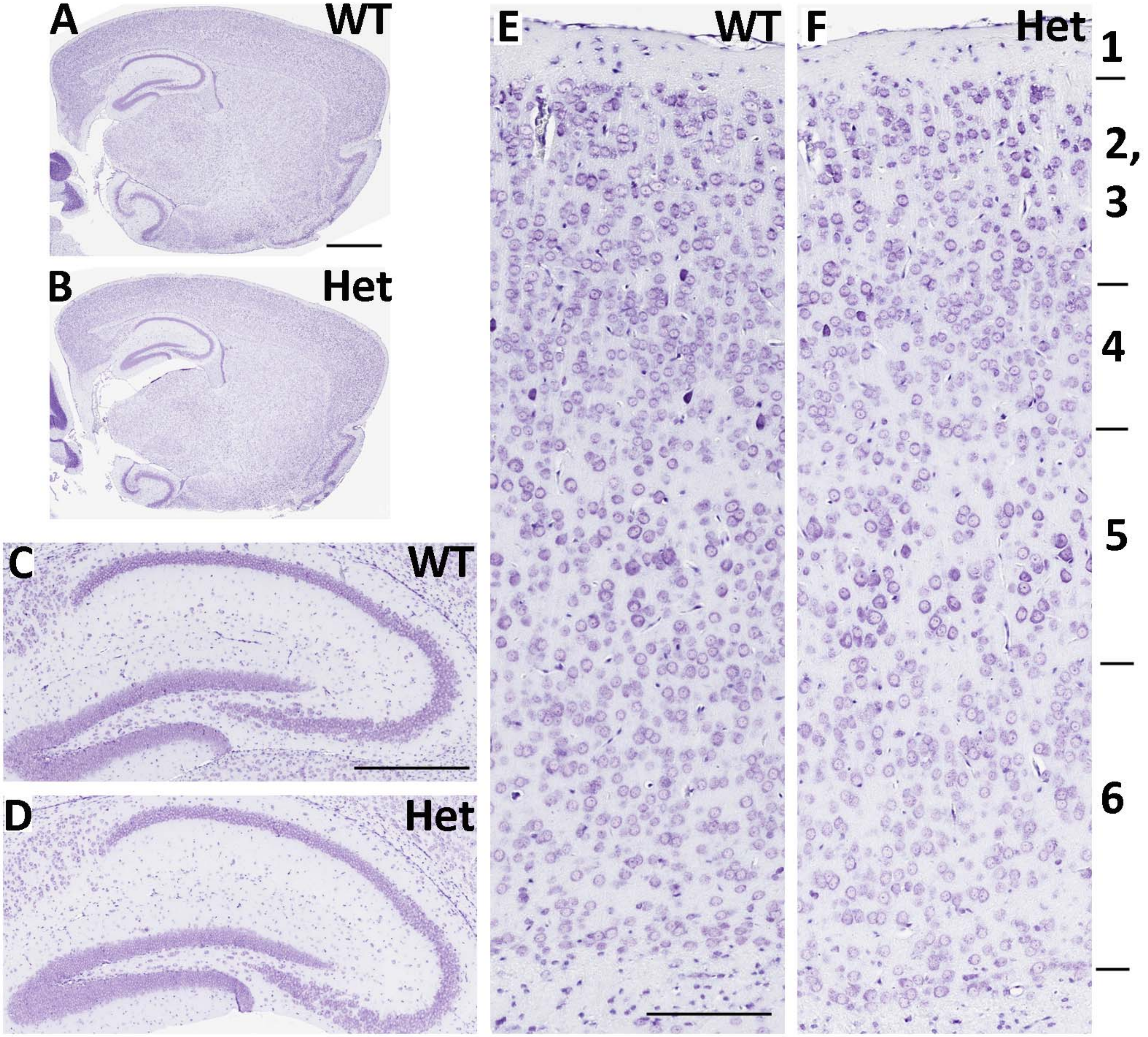


Fig S5

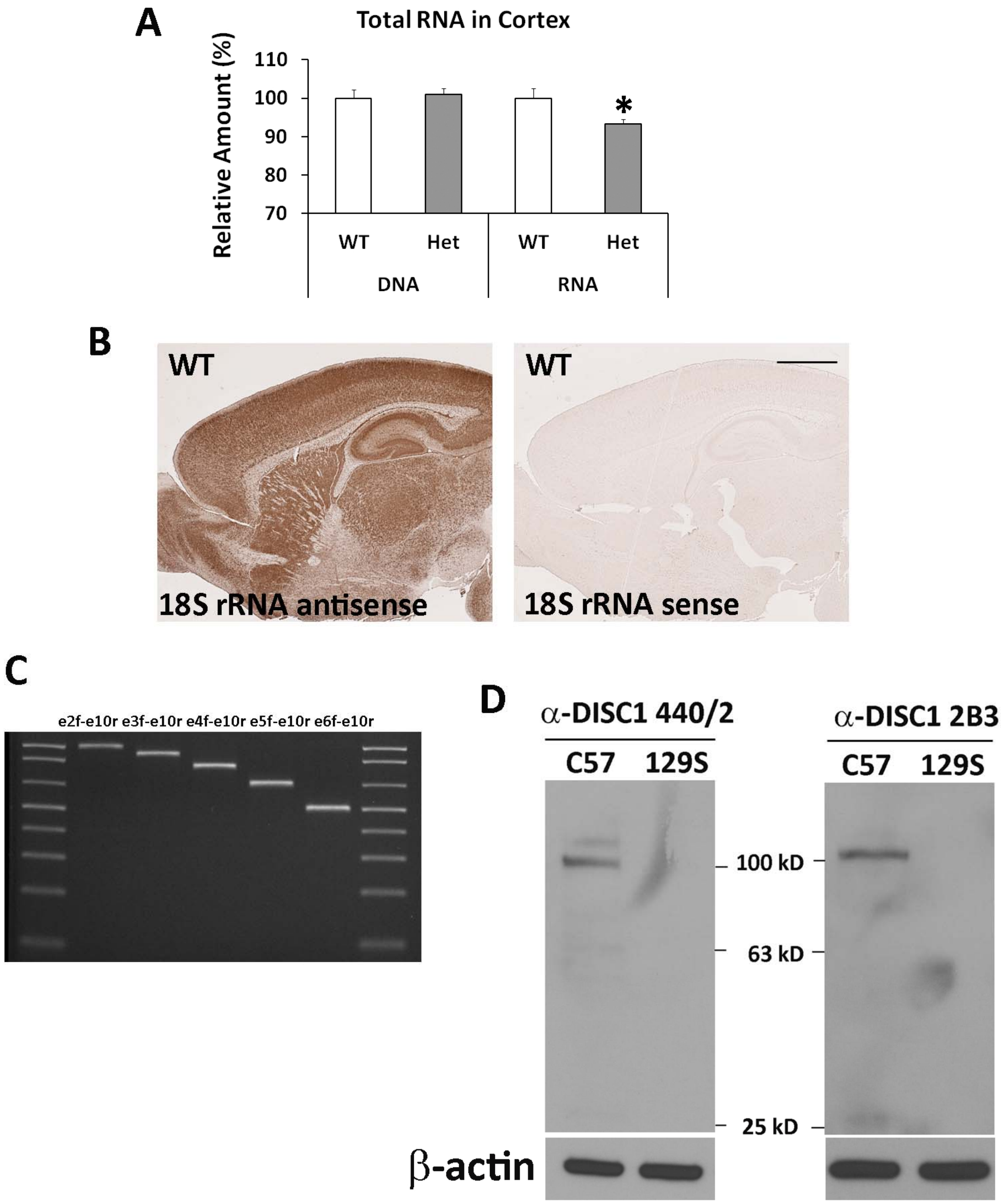

A
Total RNA in Cortex
Relative Amount (%)
110
100
90
80
70
*
WT
Het
WT
Het
DNA
RNA
B
WT
WT
18S rRNA antisense
18S rRNA sense
C
e2f-e10r
e3f-e10r
e4f-e10r
e5f-e10r
e6f-e10r
D
α-DISC1 440/2
α-DISC1 2B3
C57
129S
C57
129S
100 kD
63 kD
25 kD
β-actin

Fig S6

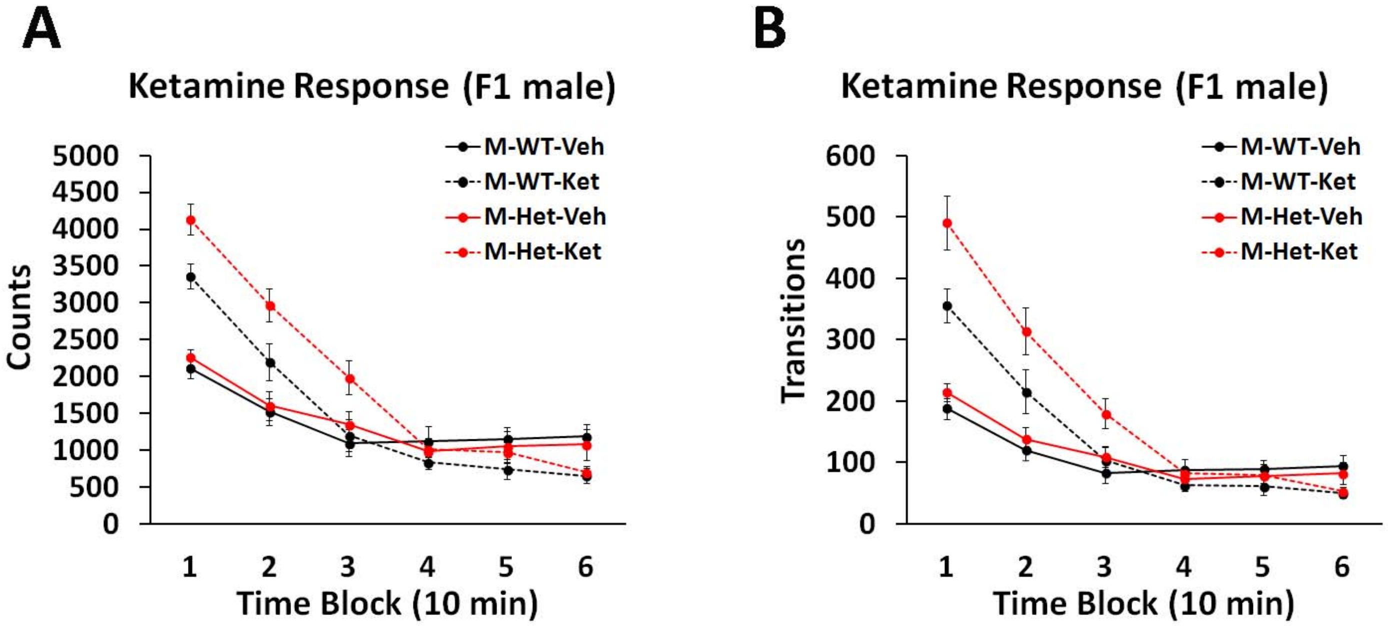

A
Ketamine Response (F1 male)
Counts
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
1
2
3
4
5
6
Time Block (10 min)
M-WT-Veh
M-WT-Ket
M-Het-Veh
M-Het-Ket
B
Ketamine Response (F1 male)
Transitions
600
500
400
300
200
100
0
1
2
3
4
5
6
Time Block (10 min)
M-WT-Veh
M-WT-Ket
M-Het-Veh
M-Het-Ket

Fig S7

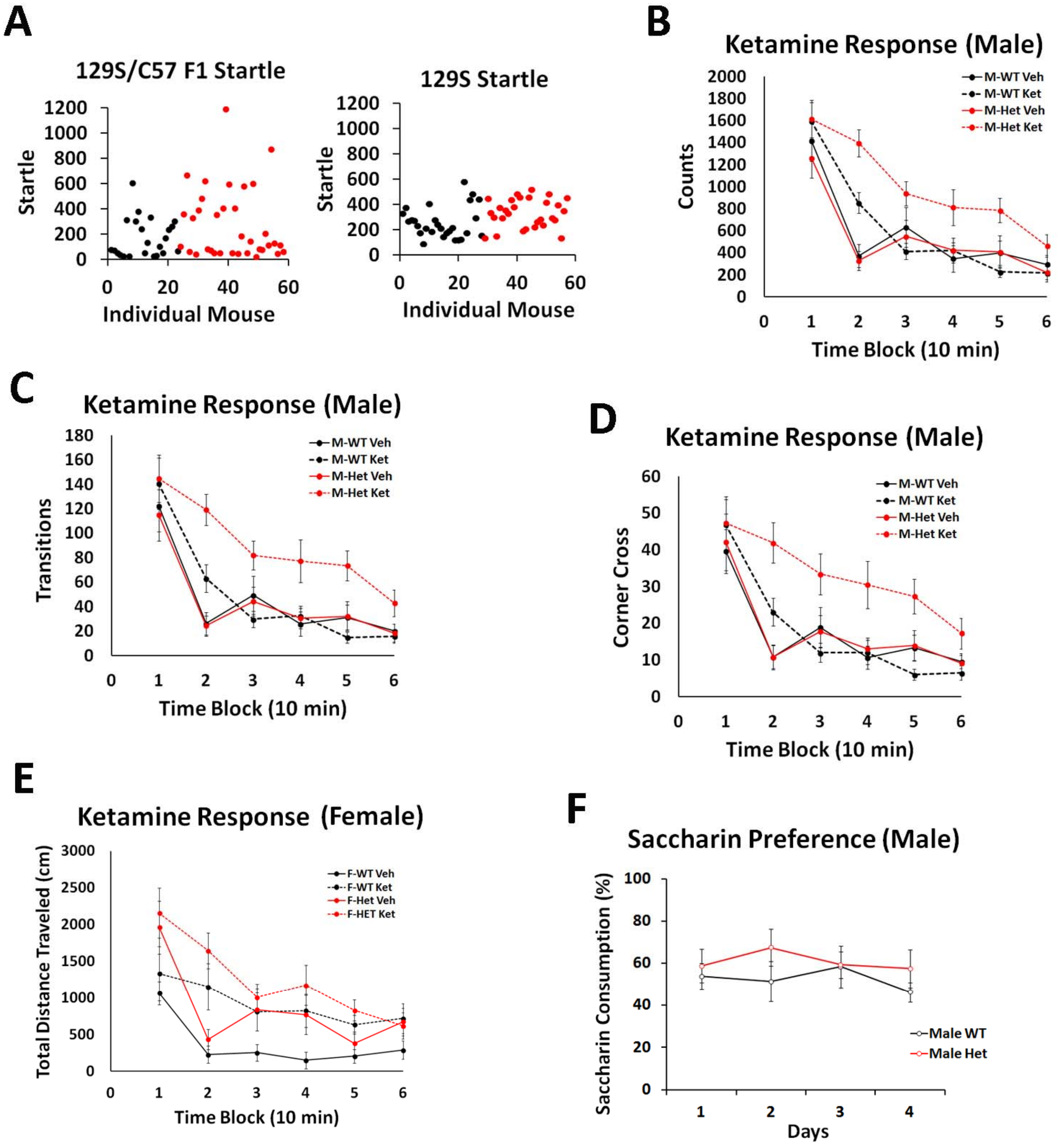

A
129S/C57 F1 Startle
Startle
1200
1000
800
600
400
200
0
0
20
40
60
Individual Mouse
129S Startle
Startle
1200
1000
800
600
400
200
0
0
20
40
60
Individual Mouse
B
Ketamine Response (Male)
Counts
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
1
2
3
4
5
6
Time Block (10 min)
M-WT Veh
M-WT Ket
M-Het Veh
M-Het Ket
C
Ketamine Response (Male)
Transitions
180
160
140
120
100
80
60
40
20
0
0
1
2
3
4
5
6
Time Block (10 min)
M-WT Veh
M-WT Ket
M-Het Veh
M-Het Ket
D
Ketamine Response (Male)
Corner Cross
60
50
40
30
20
10
0
0
1
2
3
4
5
6
Time Block (10 min)
M-WT Veh
M-WT Ket
M-Het Veh
M-Het Ket
E
Ketamine Response (Female)
Total Distance Traveled (cm)
3000
2500
2000
1500
1000
500
0
0
1
2
3
4
5
6
Time Block (10 min)
F-WT Veh
F-WT Ket
F-Het Veh
F-HET Ket
F
Saccharin Preference (Male)
Saccharin Consumption (%)
100
80
60
40
20
0
1
2
3
4
Days
Male WT
Male Het

Fig S8

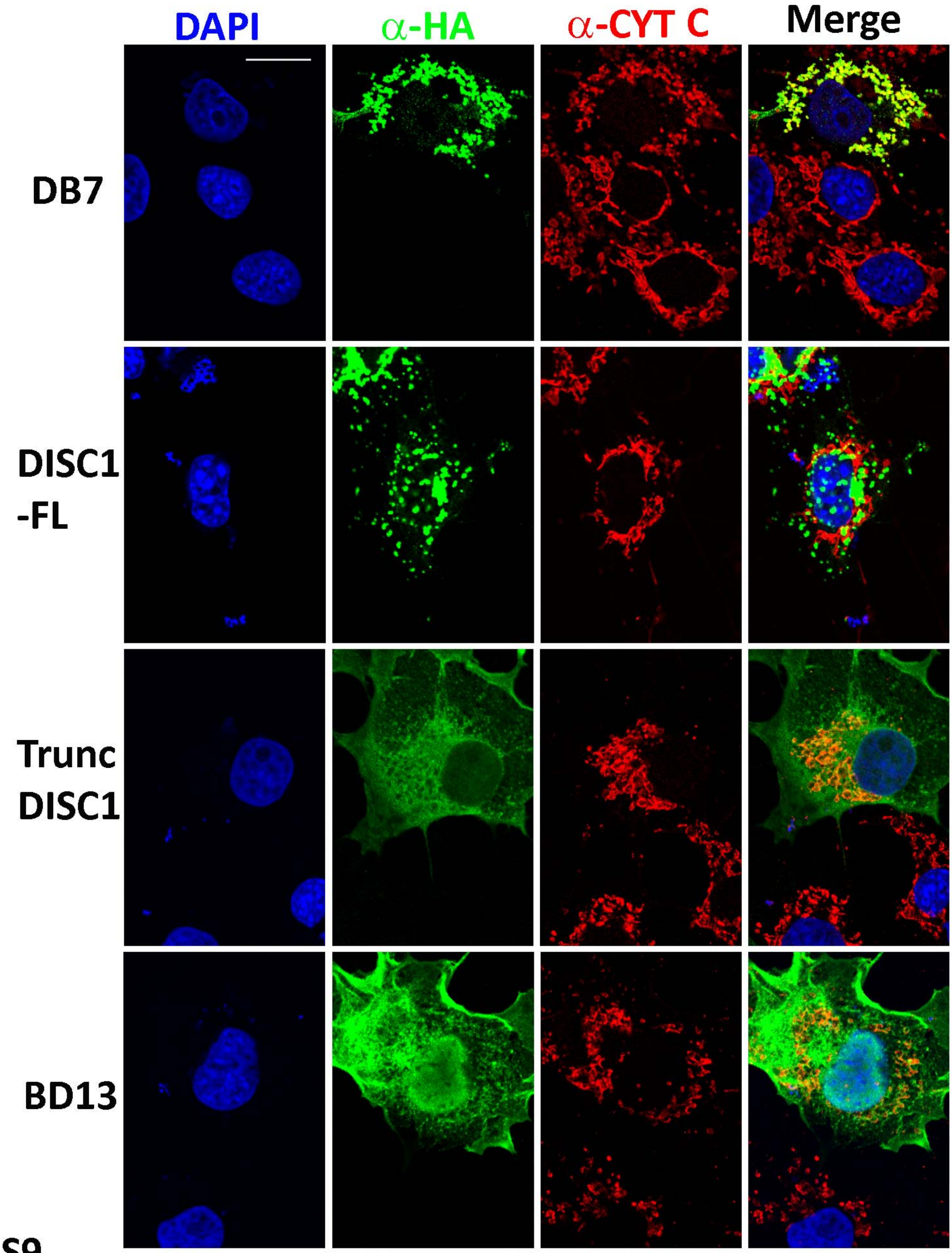


Fig S9